THE STRUCTURE OF HUMAN SKIN COLLAGEN AS STUDIED WITH THE ELECTRON MICROSCOPE*

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The application of modern methods of structural analysis in the colloidal range of dimensions to the study of connective tissue is of importance not only for the information it may yield regarding the molecular organization of this tissue but also because it may provide a means of investigating pathological alterations of the various components. Thus far, normal and abnormal mesenchymal tissues have been investigated primarily by histological and cytochemical methods. These techniques, because of their limited resolution, permit observation of changes in the fiber as a whole but cannot localize the alterations to the component collagen fibrils[1] or the interfibrillar substance.

The development of electron microscopy now permits direct visualization of tissue components of the order of 50 A in size and even smaller. However, before one can profitably investigate abnormalities it is necessary to determine the structure of normal connective tissue and to establish the range of normal variation. Two factors make this task difficult. The fragmentation techniques used to prepare specimens thin enough for electron microscopy produce certain alterations in the material which must be evaluated by studying many different samples. The samples themselves are minute, which makes a statistical approach necessary. Further improvements in methods of preparing thin sections for electron microscopy (9, 20) may minimize certain of these difficulties.

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[1] Various hierarchies of fibrous structures may be distinguished in collagen. At the level of the light microscope one sees fibers as thin as 2 μ, or, as in skin, as thick, as 20 to 40 μ. These fibers comprise bundles of still thinner units, the *fibrils*, which can be observed in teased preparations with the darkfield microscope or with the electron microscope. In certain forms of connective tissue the fibrils have fairly uniform widths (about 0.1 μ, or 1,000 A in skin) while in other types there is considerable scatter in the widths. The fibrils are presumed to be composed of bundles of polypeptide chains. These bundles which, for convenience, are called *filaments* have variable widths, from about 100 A to the limits of electron microscope resolution.

A beginning has been made in the investigation with electron microscopy of elastic tissue (29) and of one of the connective tissue polysaccharides, hyaluronic acid (10). Considerable attention has been devoted to the collagenous constituents. A periodically banded pattern was first demonstrated in electron micrographs of collagen fibrils in 1942 (11, 24). The distance between repeating bands (axial period) agrees well with that previously demonstrated by Bear (2, 3) and Kratky and Sekora (15) by x-ray diffraction methods. Wolpers (28, 30) observed the striated appearance in fibrils of osmic acid–fixed tendon, cartilage, and umbilical cord. He resolved two bands in the dense region of each period.[2] Using phosphotungstic acid (PTA) as an "electron stain," Schmitt, Hall, and Jakus (23; see also Schmitt, 22) resolved more intraperiod fine structure in rat tail tendon fibrils in the form of five bands having characteristic position and density. Six intraperiod bands were observed in mammalian skin collagen by Nutting and Borasky (19) and Schmitt and Gross (21), and as many as seven bands were observed in the axial period of fibrils in rat epineurium and kangaroo tendon (21). It seems probable that, as further technical improvements are made, still further detail of the axial repeating structure will be resolved.

Application of metal shadowing technique has demonstrated that the cross-bands represent periodic variations in thickness of the dried fibrils. Two intraperiod elevations were previously demonstrated (23, 19) but recently six elevations were resolved in platinum-shadowed fibrils of goat and calf skin, corresponding well with the relative positions of the bands in PTA-stained collagen (21).

In the present study a replica technique was used in order to permit examination of moist fibrils and to allow observation of the surface characteristics of tissue fragments too thick for transmission electron microscopy.

The primary purpose of this paper is to describe the structure observed in the collagen fibrils of normal human skin and to attempt to provide a baseline for reference in subsequent studies of collagen from pathological tissue.

Methods

Samples of abdominal skin were obtained at autopsy from 16 individuals varying in age from 1 hour to 89 years. None suffered from any known widespread disease of the connective tissue.

The unfixed skin was dissected free of readily removable fat and subcutaneous tissue, sectioned parallel to the surface with the freezing microtome, and the epidermis discarded. The frozen sections were placed in double distilled water and dispersed with needles or further fragmented in a microblendor or by sonic (9 kc.) treatment.

Some of the crude water suspensions were treated in the following manner: (*a*) Thorough washing with double distilled water in the clinical centrifuge. (*b*) Incubation in crude or crystalline trypsin (0.1 per cent in isotonic saline or distilled water) for 24 hours at 37°C. The suspension was then washed in double distilled water. (*c*) Incubation in hyaluronidase,[3] in varying concentration up to 20 mg. per cent in water or isotonic saline at 37°C. for 2 hours, followed by washing.

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[2] In a personal communication Wolpers described four bands in the dense part of the period.

[3] This preparation of testicular hyaluronidase was kindly furnished by The Schering Corporation.

Specimens were prepared for electron microscopy by placing a drop of suspension on the usual collodion or formvar supporting film of the specimen grid. In a few cases frozen sections were smeared onto specimen films without preliminary fragmentation.

When staining was desired the specimen grid was drained and a droplet of PTA solution (0.1 to 1.0 per cent in phosphate buffer at pH = 5) applied. The grid was inverted, to avoid deposition of unwanted suspended material and staining allowed to proceed for periods varying from 15 seconds to 5 minutes. The preparation was then washed by immersion in double distilled water, with gentle agitation, for about 15 seconds and air-dried.

For metal shadowing, collagen fibrils were deposited on a film-covered grid, air-dried, and placed in an evaporation chamber where they were subjected to a beam of metal atoms impinging at angles varying from 6 to 20°, after the method of Williams and Wyckoff (26). Chromium or platinum were usually used; in the former case the calculated thickness of deposited metal was about 15 A normal to the film, in the latter case 4 to 6 A.[4]

The technique of preparing thin plastic films, described by Shaefer and Harker (25), was modified for use in preparing replicas. Various combinations of plastics and solvents were used. These included collodion or methyl methacrylate in secondary butyl acetate, formvar in ethylene dichloride, and polystyrene in benzene. A 0.2 per cent solution was found to provide optimum film thickness.

A water suspension of fragmented connective tissue was air-dried on a clean glass slide. With a very thin film of moisture still present on portions, the slide was plunged into the plastic solution, allowed to remain for 5 to 30 seconds, then dried in the vertical position in air. The moist patches could be seen beneath the dried plastic. The film was then sectioned into squares with a needle and floated off onto a clean water surface. These portions of film were then transferred to nickel grids and shadowed with chromium in the usual manner in order to increase contrast. Other glass slide preparations were completely desiccated in a vacuum chamber before applying the plastic. Frozen sections of tissue were smeared directly onto the glass slide and replicas prepared therefrom. A similar technique has been used for the study of bacterial and bacteriophage colonies on the surface of agar (7, 14). Claude (4) has also used the method for the examination of air-dried blood cells.

An RCA type EMB electron microscope was used in this study. All measurements of intraperiod band positions were made with a microdensitometer from the original electron micrographs.

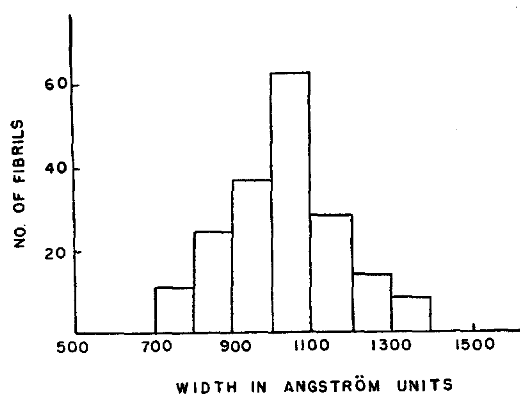
RESULTS

When frozen sections of human skin, cut parallel to the surface, are fragmented in water the solution becomes opalescent due to the release of collagen fibrils in various states of aggregation from single fibrils to bundles of fibrils. Viewed in the darkfield microscope the fibrils from adult skin appear as smooth, relatively straight, unbranched threads many microns in length. The fibrils of corium from infants are much more cohesive as judged by the amount of fragmentation required to disintegrate the tissue. In the darkfield these fibrils are more frequently clumped and appear to be finer, shorter, and more cortorted than are those of adult skin.

[4] Although the high temperatures required for evaporation of platinum may affect the specimen, this metal is preferable to chromium because of the thinness of the layer necessary to give the desired contrast; moreover, it does not aggregate in the electron beam as does gold. Evaporation of platinum is facilitated by the addition of 1 part of palladium to 4 parts of platinum, as suggested by Williams and Bachus.

In Fig. 1 is shown an electron micrograph of a group of fibrils from an incompletely fragmented tissue section. In such bundles of fibrils the cross-bands are frequently in lateral register. It is probable that this type of organization is characteristic of intact, normal tissue.

Fibril Widths.—Limitations of resolution make it difficult to measure widths of individual fibrils with an accuracy greater than about 50 A. However, the collagen fibrils of different forms of connective tissue vary considerably in range and uniformity of widths. In Text-fig. 1 is shown a distribution curve of widths measured on 180 fibrils of adult human skin. Widths range from about 700 to 1,400 A, the majority being about 1,000 A. Fibrils from infant skin show widths as small as 300 A though the average is not significantly different from that of adult skin. Wolpers (27) stated that the fibrils are



TEXT-FIG. 1. Distribution of fibril widths (180 fibrils measured)

thinner in young than in old individuals. The widths of fibrils of rat tail tendon range from almost as small as can be resolved by the electron microscope to 2,000 A and more (24). This variation probably reflects a real difference in the organization of the fibrils in different tissues rather than alterations due to the method of preparation.

Axial Repeating Period.—A distribution curve of values of the axial repeating period of skin fibrils was constructed. At least 10 consecutive periods in each of 200 chrome-shadowed fibrils were measured. As shown in Text-fig. 2, the periods ranged from about 500 to 800 A, the maximum occurring between 620 and 660 A. This distribution is similar to that found by Nutting and Borasky (19) in cow skin fibrils; the range of variation is somewhat less than that found for the collagen of rat tail tendon by Schmitt, Hall, and Jakus (24). The average period in fibrils of adult skin does not appear to differ significantly from that of fibrils from infant skin.

Axial Intraperiod Structure.—Unstained skin fibrils usually show little detailed intraperiod structure but resemble that shown in Fig. 3. In rare in-

stances as many as five intraperiod bands were distinguished. It is possible that, with different technique, fine structure may be resolved with more regularity. However at present, staining is required to bring out detailed fine structure in most preparations.

Even after staining with PTA the fraction of fibrils showing more than two or three dense bands per period is small. This is probably due not to a deficiency in the resolving power of the electron microscope but rather to imperfections of organization of the fibrils resulting from distortions introduced during preparation of the specimen. Wolpers (27) believed that removal of ground substance by sonic treatment is required for visualization of fine structure. However, in the present studies, the highest proportion of fibrils showing detailed intraperiod fine structure was found in a specimen in which the fibrils were embedded in an amorphous matrix which could not be removed by water washing. While such amorphous material undoubtedly reduces resolution of fibril structure, the primary factor in observing fine structure is probably distortion of the lateral relationship of the filaments within the fibril.

The most characteristic intraperiod band pattern observed in well preserved fibrils is that containing six unequally spaced bands as shown in Fig. 5. This pattern has been observed in fibrils of animal skins, tendon, and nerve epineurium (21). The bands have been tentatively termed: a, b_1, b_2, c, d, and e. The a band, which is located at the widest part of the period in cases where the fibril shows a scalloped appearance, is the broadest and most dense of the six bands. In a few cases the a band appeared as a doublet. The adjacent b_1 and b_2 bands have the smallest separation; with poorer resolution these appear as a single band, b, as previously observed in rat tail tendon fibrils (22).[5] Fig. 4 illustrates this pattern in a skin fibril stained with 0.1 per cent PTA. The d band is characterized by its relatively high density in contrast to adjacent regions. The c band is not sharply defined, probably because it represents several unresolved, fine bands; two of these subdivisions were observed in a somwhat stretched kangaroo tendon fibril. The e band is almost always diffuse and of relatively low density.

The average separation between bands, is about 18 per cent of the total period except for the b_1-b_2 separation which is about 11 per cent. These values agree well with those observed in fibrils of chrome-tanned calf and goat skin by Schmitt and Gross (21). They are essentially constant in the range of periods for which measurements were made (500 to 900 A).

It is probable that, as improvements are made in methods of preparation of the material and in the imaging ability of the electron microscope, still further

[5] There is some indication that conditions of staining may be important in resolving these bands. Successful resolution occurred when 1 per cent PTA was used whereas, when the PTA concentration was 0.1 per cent, only one band in this region (b) was observed. There is a possibility, however, that the five-banded pattern is an actual entity and represents another form of collagen.

details of intraperiod structure will be resolved. The present observations were made with the through-focus technique; *i.e.*, a series of micrographs was made of each specimen at different focal levels. This not only facilitates obtaining the best definition but also permits evaluation of diffraction effects characteristic of such finely banded structures.

Chromium- or platinum-shadowed fibrils show two characteristic intraperiod elevations as a rule. However; additional fine structure is frequently visible and, in the most favorable cases, six intraperiod elevations are seen (Fig. 7). These elevations correspond in position with the six bands observed in stained fibrils.[6]

From this evidence it appears that the cross-striations are regions which are relatively thicker than the interband regions. This suggests that the contrast produced by staining with heavy metal salts could be due in part to a volume effect as well as to a specific chemical combination of stain with groupings in the collagen molecules. Nutting and Borasky (19) favored the former suggestion.

Lateral Structure in Fibrils

It has been shown that the collagen fibril is composed of very thin filaments of varying widths ranging from about 100 Angström units down to and below the limits of resolution of the electron microscope. The filaments have been directly visualized in acetic acid "solutions" of rat tail tendon and fish swim bladder ichthyocol.[7]

In shadowed human skin fibrils, particularly when the beam of metal atoms impinges on the fibril at right angles to the fibril axis, a fine longitudinal filamentous structure can be observed (Fig. 8). From three to ten such filaments may be visible in different regions of the same fibril. The filaments pursue a predominantly longitudinal course and frequently may be followed individually through a number of periods. Where they interesect the cross-bands there is is a nodose appearance.

Such filaments may well be aggregates depending on factors involved in preparation, chiefly those operating during drying. However, they probably represent potential cleavage planes. In rat tail tendon the filamentous nature of the fibril is readily demonstrated because of the tendency of these fibrils to cleave longitudinally when fragmented. Skin fibrils seldom show such cleavage; initial fracture usually occurs in directions roughly normal to the axis. A fraction of the observed severed ends represents fibrils which were cut by the microtome. However, we have observed many cases such as that illustrated in Fig. 9. Here may be seen the fractured end of a fibril with the replica imprint of the lost fragment, showing that the fracture occurred after the fibril

[6] While it is difficult to reproduce such electron micrographs, the positions of the bands are readily determined with the microdensitometer.

[7] Unpublished data.

was deposited on the slide. Occasional disruption of a fibril has been observed but no true fray has been seen. The disorganized regions appear amorphous instead of filamentous as does rat tail tendon.

In a number of micrographs there have been observed ellipsoidal, disc-like objects having diameters approximately equal to those of the fibrils. It is possible that these represent thin sections of fibrils, possibly one or several of the repeating periods, removed during fragmentation. This idea is supported by the fact that, in occasional instances, entire periods were missing along the lengths of fibrils, or were seen to be displaced from the fibril axis.

Where the fibril adheres firmly to the plastic film it may flatten to a thin ribbon on drying (Figs. 6 and 9). Nevertheless, the axial repeating pattern is usually as well preserved in such flattened fibrils as in those which dry with a more circular or elliptical cross-section. Apparently the filaments which compose the moist fibril are relatively free to rotate about each other without loss of regular axial repeating structure and whilst maintaining considerable interaction with each other laterally, so that the fibril is uniformly cross-striated.

Information about drying patterns is given also by shadowed specimens. In Fig. 7 is seen a fibril shadowed at an angle oblique to the fibril axis. The wedge-shaped shadow at the lower left region of each period demonstrates the cylindrical character of the fibril. When the fibril flattens to a ribbon on drying this feature is not present and shadows are very short or absent. The ribbon-like character of the fibril in Fig. 6 is further indicated by the wrinkling at the right end, produced by bending in a direction parallel to its flat surface.

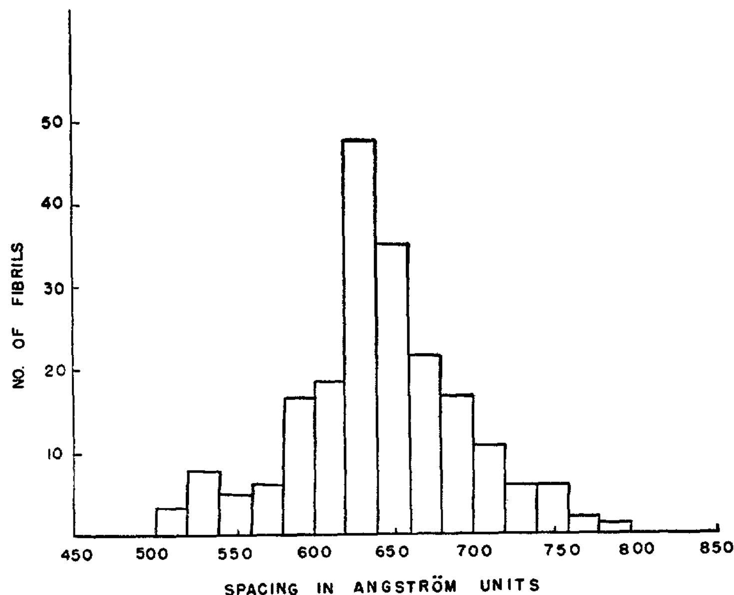
Replica Studies

Replicas of Dried Fibrils.—In the preparation of replicas of collagen fibrils deposited on glass slides many of the fibrils are removed with the plastic replica film. These may be found embedded in the plastic film or somewhat displaced from their imprints as a result of tensions during stripping (Fig. 2). This fortunate circumstance permits comparison of the structure of the dried fibril with that of its imprint in the replica. In the majority of cases the fibrils remain on the glass slide and only their imprints are seen.

The axial repeating period is well shown in replicas. The distribution curve of 100 imprints resembles closely that obtained directly from the fibrils (Text-fig. 2). The best replicas show two and three intraperiod bands (Fig. 11) but the definition is not good enough to permit measurement of positions. In rare cases four bands may be seen.

The elevations in the replicas represent depressions (thin regions) in the collagen fibril. This information about the contour of the intraperiod bands of dried fibrils supplements that obtained from the shadowed fibrils themselves. This contouring is best observed when the metal atoms fall upon the replica in a direction parallel with the fibril axis during shadowing.

The solvent used for dissolving the plastic may be of importance in this technique. The replicas of some dried fibrils prepared with ethylene dichloride as solvent for formvar were considerably wider than the fibrils themselves and in many cases little axial repeating structure was shown. The possibility that the solvent may have reacted with the collagen in such instances is being fur-



TEXT-FIG. 2. Distribution of axial periodicity (200 fibrils measured)

ther investigated. Fig. 2 represents an unusually good formvar replica of dried fibrils. Collodion in sec-butyl acetate has proved most satisfactory to date. Fig. 10 is a good example of a dried collodion replica of collagen bundles.

Replicas of Moist Fibrils.—It is clear from x-ray studies (3) that drying alters the structure of collagen in a characteristic fashion. Electron microscopy of collagen fibrils is possible only in the dried state. However, information concerning the structure prior to drying may be obtained from replicas of moist fibrils.

Replicas of moist fibrils resemble impressions made in smooth sand.[8] In Fig. 12 (collodion replica) two crossed fibrils were transferred from the glass with the film. A faint periodic contouring, corresponding to the axial repeating pattern, is usually seen in the imprints. However, in some cases this is almost completely lacking, indicating that the surface of the moist fibril may be essentially smooth.

The widths of replicas of moist fibrils are usually somewhat greater than those

[8] In our hands formvar in ethylene dichloride produces unsatisfactory, coarse-grained replicas of moist specimens, probably because of poor spreading on wet surfaces.

of the fibrils themselves and the edges are poorly defined. This may be due to the tendency of the moist fibrils to flatten and adhere firmly to the glass surface.

Replicas were readily prepared from smears made by gently rubbing fresh tissue sections on a clean glass surface. Fig. 13 shows a replica of a group of fibrils obtained in this manner. The axial repeating pattern is well represented as are some details of structure. Apparently these fibrils were aligned with periods in lateral register. Similar micrographs have been obtained from the skin of newborn as well as aged individuals. This technique, which is readily applicable to biopsy specimens, is particularly useful in studying the relationship of the various tissue components and is thus a valuable supplement to methods of fragmentation and sectioning.

In all aqueous suspensions of fragmented connective tissue considerable amounts of amorphous material were present, the largest quantity occurring in infant skin where it almost completely obscured the axial periods of the collagen fibrils. Even after thorough washing in double distilled water this material persisted to a greater or lesser degree in most specimens. Replicas of smears made directly from the frozen sections of both adult and newborn infant skin produced imprints of fibrils which showed about as much detailed axial structure as did those of well washed collagen (Fig. 13). This suggests that some at least of the fibrils in the intact adult and infant corium are not closely invested with a layer of adherent interfibrillar ground substance. Smears of the fresh tissue made directly onto the supporting film of the specimen grid and shadowed with chromium were usually too thick for satisfactory study. However in a few instances they showed some detail of structure (Fig. 14), These fibrils were embedded in considerable amounts of apparently structureless material, although their free surfaces were relatively clean.

Miscellaneous Observations

In preliminary experiments no action of trypsin and hyaluronidase on the collagen of infant and adult skin was observed. However, different methods will have to be devised to investigate the action of enzymes, particularly on the amorphous matrix, because the products of the reaction cannot be differentiated from other amorphous material usually found in such preparations.

In all the preparations of connective tissue studied in these experiments the paucity of formed constituents other than collagen fibrils and poorly defined amorphous material was noteworthy.

Occasionally structures resembling elastic tissue elements, as described by Wolpers (29), were observed.

Although histological examination of infant skin, from the same specimens studied with the electron microscope, showed argyrophilic fibers characteristic of reticulin, no differentiation between collagen and reticulin fibers was obvious in the electron microscope.

In some specimens of infant skin occasional smooth edged filaments, about 100 A in width and of variable length, were noted. These occurred as individual filaments and as bundles of filaments. Their nature is at present not clear.

One grid, prepared from a shadowed replica of fragmented skin, bore no typical collagen fibrils but showed numerous, broad, double edged, striated fibrils surrounded by dense, adherent amorphous material. Except for considerably greater widths, these fibrils are suggestive of the "neurotubules" described by De Robertis and Schmitt (6). Possibly these structures derived from cutaneous nerves or their terminal twigs.

DISCUSSION

From the results obtained with replicas of moist fibrils it appears that the collagen fibril, as it exists *in vivo* is essentially a smooth contoured cylinder or ellipsoidal ribbon. Removal of water, in drying, gives the fibril a contour whose details conform to those of the banded structure seen in stained or shadowed fibrils, possibly resulting from differential shrinkage of highly localized regions within the repeating period. Obviously the structure observed in any particular collagen fibril, whether from normal or abnormal tissue, depends upon the internal adjustments of the fibril during the processes of tissue fragmentation, staining or shadowing and, finally, a very thorough drying. This internal adjustment probably concerns the interrelationship of the filaments thought to compose the fibril. It has been emphasized above that the range of variability of intraperiod fine structure observed in fibrils from one particular normal tissue, the collagen of human skin, is great. It is possible that most of these variations depend on alterations produced by the preparative procedure, as mentioned above. However, it is also possible that inherent structural variations exist, not only as a function of type of connective tissue and of age, but also within closely delimited regions of the same tissue. In this case the difficulties of the electron microscope approach to the problem become greatly multiplied because of the necessity of sampling many regions of the specimen. This is true in respect to the details of structure which require the highest available resolution. Possibly valuable information will come, at lower resolutions, from examination of thin sections of connective tissue prepared by improved microtome techniques.

With these facts in mind it may be useful to indicate the detailed structure which appears best to characterize the collagen fibrils of normal human skin, as thus far investigated.

A large axial repeating pattern in the form of cross-bands is a characteristic shared with other fibrous proteins such as fibrin (13), keratin (8), paramyosin (12), and "neurotubules" (6). Nor is the range of spacings (500 to 800 A in the present material) unique to collagen; it is essentially similar to that of neurotubules. The particular six banded pattern shown in Figs. 5 and 7 is,

so far as we know, characteristic of collagen, having been found in skin, kangaroo tendon, and rat epineurium. It is possible that, as more detailed intraperiod structure is resolved, the "collagen pattern" will be defined more exactly.

It should be pointed out here that, as Astbury (1) has shown from x-ray results, there is probably a class of collagen-like proteins, all having the property of manifesting the wide-angle x-ray pattern characteristic of vertebrate collagen. These proteins differ markedly in amino acid composition from vertebrate collagen and are obtained from animals as low in the phylogenetic series as the sponges. They also have an axial repeating period similar to that of vertebrate collagen though there are differences of intraperiod structure.[9] Accordingly, it is possible that any protein belonging to the collagen class will show a banded structure in electron micrographs and that identification will depend on differences of detail.

Collagens differ also in respect to properties of the fibrils in the lateral direction. As brought out in the present experiments, the fibrils of skin do not show the longitudinal cleavage characteristic of tendon fibrils; mechanical fracture usually occurs normal to the axis. This suggests that the lateral bonding between filaments in the fibril may be considerably stronger in skin than in tendon fibrils (16). Strong lateral bonds between the main chains, contributed by the protein itself or by other substances, could produce a great increase in lateral cohesion. Careful quantitative studies of the strength of the various linkages in collagen are lacking. Such information might provide criteria as valuable for the characterization of collagen as structural data.

There is some evidence of lateral affinity between adjacent fibrils. It has been frequently observed, in bundles of closely adherent fibrils, that the fibrils are in nearly perfect register with respect to the cross-bands. Whether this is due to specific affinities of chemical groups at various levels in the periods or to some undefined binding material is not clear. It seems probable that this interfibrillar integration may play an important rôle in the mechanical properties of connective tissue.

In current studies it has been assumed that the fibrils possessing the structural characteristics described above are primarily composed of the protein collagen. It has been suggested that collagen is closely associated with other components such as polysaccharide (17) and protein (5). In the present experiments efforts have been made to test this view but the results are inconclusive. No clear-cut effects have been observed as a result of exposing skin collagen fibrils to the action of hyaluronidase. However, certain polysaccharides are insensitive to the testicular hyaluronidase used; if such be present, bound to the collagen as Meyer (18) suggests, no positive result would be expected in the above experiment.

Similar considerations apply to the experiments involving the effect of trypsin. This enzyme has been used frequently to rid preparations of non-

[9] From unpublished data of M. H. Marks, R. S. Bear, and C. H. Blake.

collagenous proteins and thus better to reveal the structure of the collagen. The usual detailed fine structure has been observed in such enzyme-treated material. However, this is not proof of the absence of a non-collagenous protein in the fibril nor yet of the complete insensitivity of all collagen to tryptic attack. Steric effects and mode of packing might be responsible for the insensitivity. The subject of the action of proteolytic enzymes on collagen is a large and important one which warrants detailed study.

The present experiments throw little light on the nature of the amorphous ground substance in which the collagen fibrils are thought to be embedded in connective tissue. While purified hyaluronate has a characteristic morphology (10), this is closely dependent on methods of preparation and might well be masked in the present experiments, which involved extensive fragmentation and water washing. Possibly the replica-smear technique will prove valuable in the study of the amorphous component.

SUMMARY

1. The structure of the collagen fibrils of normal human skin corium has been investigated with the electron microscope.

2. Under the conditions of observation the fibrils ranged in width from about 700 to 1,400 A with 1,000 A as the value occurring most frequently. They showed little tendency to fray longitudinally as is characteristic of tendon fibrils; when fracture of fibrils occurred it was usually in planes transverse to the axis.

3. The axial repeating periods observed in fibrils stained with phosphotungstic acid or shadowed with chromium or platinum range from about 500 to 800 A, the maximum occurring between 620 and 660 A. The average period in fibrils from infant skin does not differ significantly from that of adult and aged skin.

4. Depending on conditions of preparation, intraperiod fine structure, in the form of cross-bands, was observed in varying detail. The most detailed pattern commonly observed contains six bands of characteristic density and position.

5. Shadowed plastic replicas of dried collagen fibrils reproduce much of the structure commonly seen in shadowed fibrils. Replicas of moist fibrils show considerably less surface contouring than do dried fibrils. Replicas from smears of connective tissue fragments on glass show detailed structure, indicating the feasibility of applying this technique to biopsy material.

6. Infant skin differs from adult skin in having considerably greater amounts of amorphous material, little of which is strongly adherent to the collagen fibrils.

Addendum.—Since submission of this manuscript we have received, through the courtesy of Dr. C. Wolpers, two papers by this author, not previously available to us (*J. Makromol. Chem.*, 1948, **2**, 37; *Biochem. Z.*, 1948, **318**, 373). Working chiefly with osmic acid fixed beef tendon, Wolpers studied the effect of thermal shrinkage and of acid treatment, as well as certain pathological conditions (rabbit virus myxoma) upon the detailed structure of the collagen fibril. The normal fibril, he finds, is characterized by the presence of two dense (δ) bands per period. After heating or other treatment four bands (δ lamellae) are observed. In the transformation from two to four bands he suggests that a cementing material ("*Kittsubstanz*") of unknown nature

is removed and "δ" particles make their appearance in the fine bands. Further heat treatment destroys the banded structure and leads to an irregular filamentous network which, when fully dissociated, produces glue or gelatin.

Wolpers' view that detailed intraperiod structure (more than two bands) is the result of alteration or degradation of the structure of normal collagen is in contrast to that expressed in the present paper, in which it is supposed that the normal fibril is possessed of very detailed intraperiod structure which may not be fully resolved because of various factors operative during the preparative procedure. In this connection it may be pointed out that detailed intraperiod structure (six bands) has been observed in material which had been subjected only to teasing with needles and brief water washing, followed by PTA staining. It seems improbable that such mild treatment could have caused the alterations visualized by Wolpers as responsible for the production of the multibanded structure.

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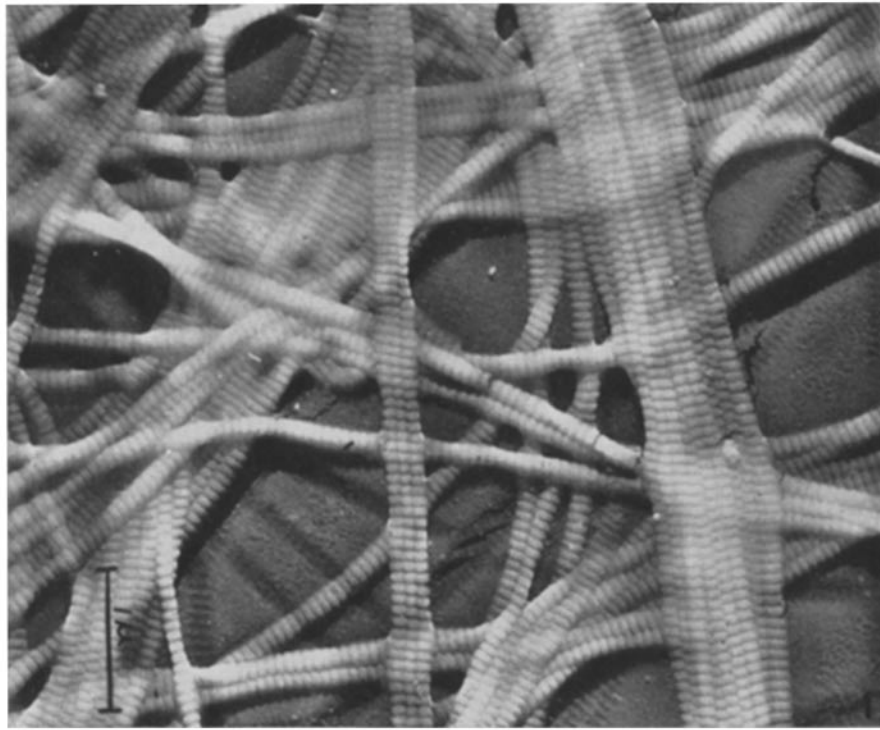
EXPLANATION OF PLATES

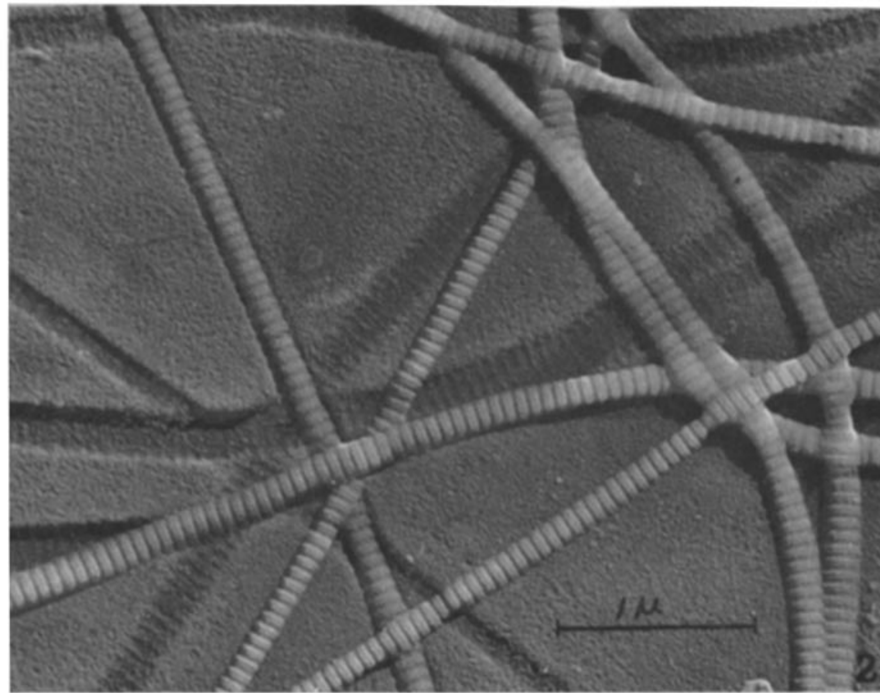
PLATE 29

Formvar replica of collagen fibrils from adult human corium deposited on a glass slide from aqueous suspension and air-dried. Shadowed with chromium.

FIG. 1. Fiber bundles adherent to film. In background a few imprints are seen. × 19,300.

FIG. 2. Individual adherent and embedded fibrils and imprints. × 26,000.





(Gross and Schmitt: Human skin collagen studied with electron microscope)

PLATE 30

Adult corium fibrils from fragmented frozen sections washed thoroughly in water and deposited on a specimen grid from suspension.

FIG. 3. Stained with 0.1 per cent PTA. Most unstained and many stained fibrils have this appearance in which little or no intraperiod structure is present. × 95,000.

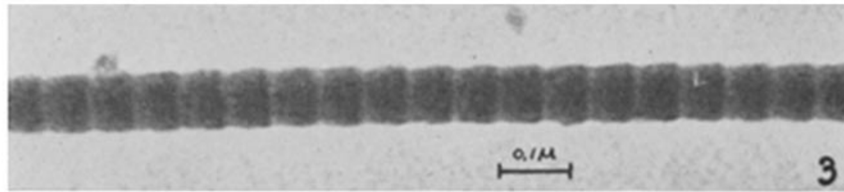
FIG. 4. Stained with 0.1 per cent PTA. Five bands resolved. × 100,000.

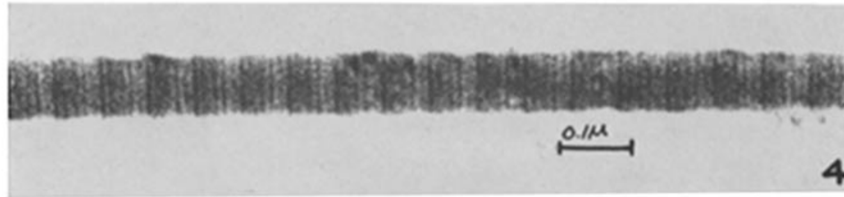
FIG. 5. Stained with 1.0 per cent PTA. Six bands resolved. × 100,000.

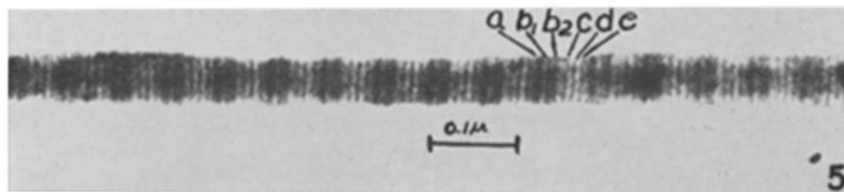
FIG. 6. Flattened fibril showing four and in some periods five bands. Note kinking of ribbon at right end. Shadowed with chromium. × 106,000. (Arrow indicates direction of shadow.)

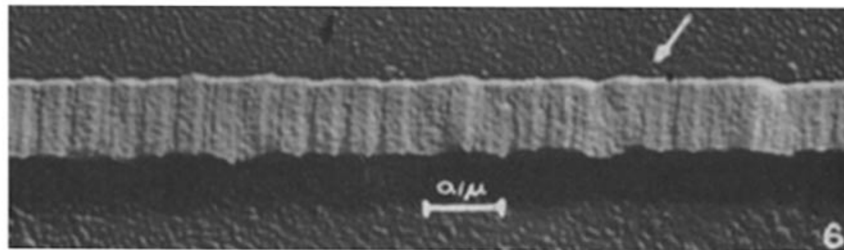
FIG. 7. Fibril showing six-banded period. Shadowed with chromium. × 80,100. (Arrow indicates direction of shadow.)

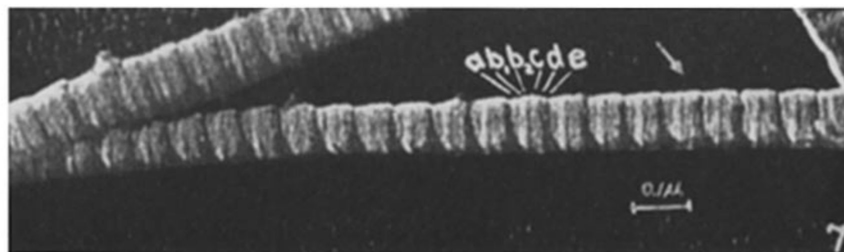
FIG. 8. Longitudinal aggregates of filaments are seen in this fibril shadowed with chromium. × 98,000. (Arrow indicates direction of shadow.)

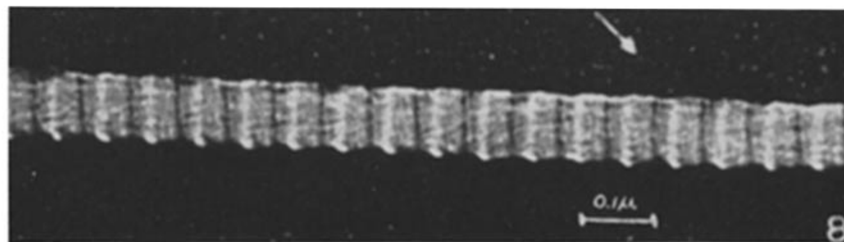










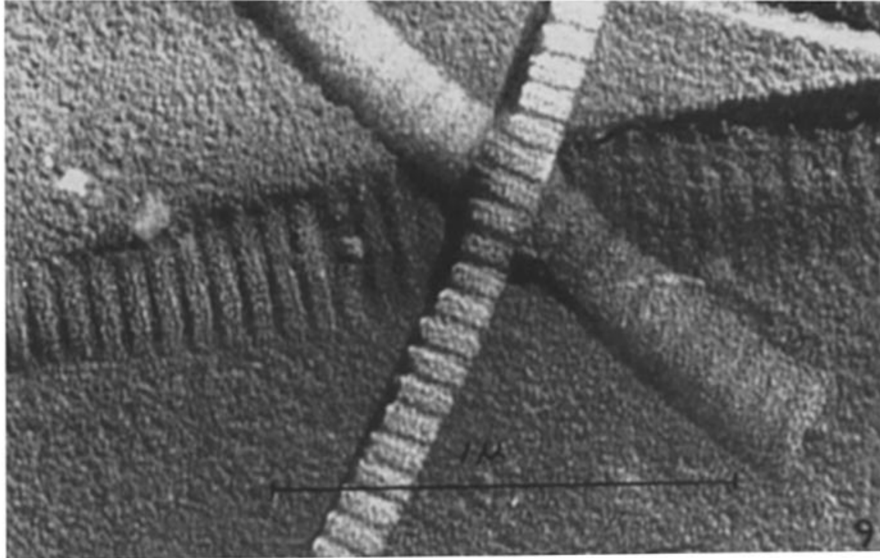


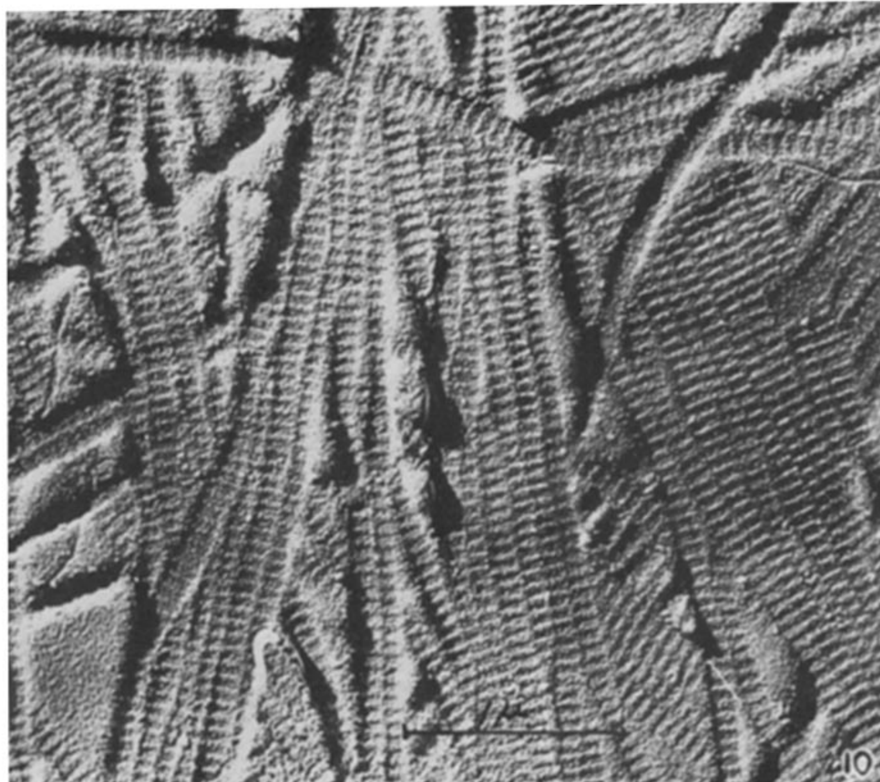
(Gross and Schmitt: Human skin collagen studied with electron microscope)

PLATE 31

FIG. 9. Chromium-shadowed formvar replica of air-dried fibrils deposited from suspension on glass slide. Note ruptured fibril at left with imprint of remainder; two other adherent fibrils are shown. × 61,500.

FIG. 10. Collodion replica of vacuum-dried fibril bundles. Deposited on glass slide from water suspension. Shadowed with chromium. × 28,500.





(Gross and Schmitt: Human skin collagen studied with electron microscope)

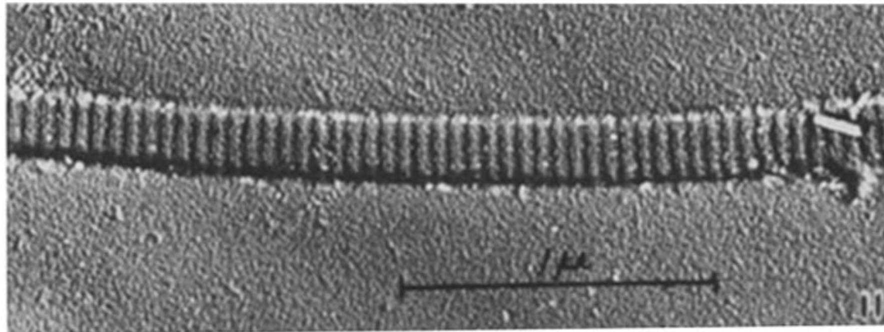
PLATE 32

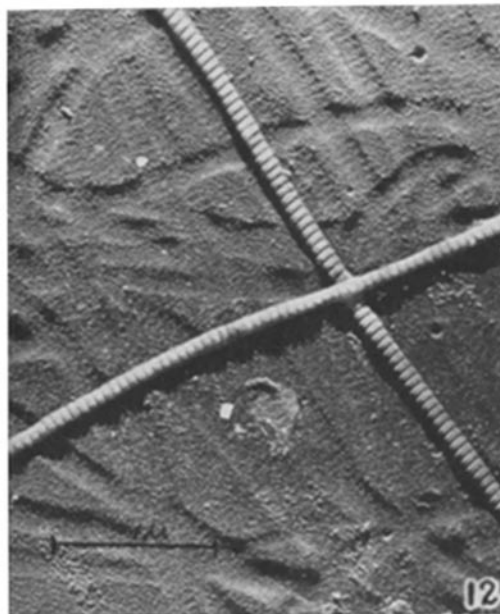
FIG. 11. Chromium-shadowed collodion replica of air-dried fibril showing three intraperiod bands. Deposited on glass slide from water suspension. × 42,000.

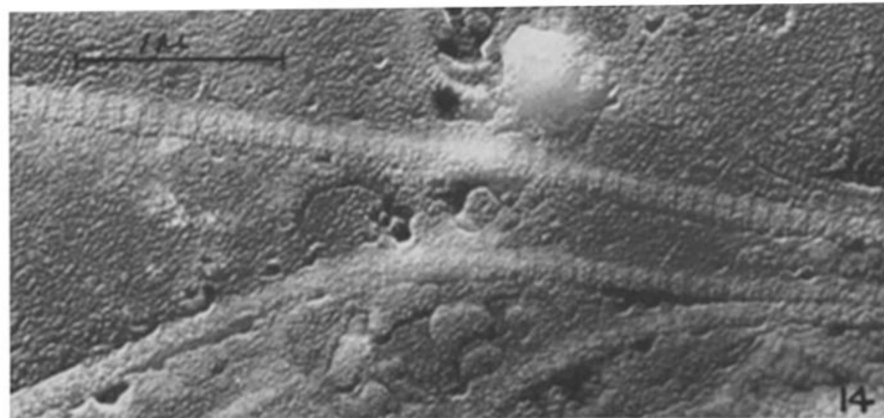
FIG. 12. Chromium-shadowed collodion replaca of moist fibrils deposited on glass slide from suspension. Two adherent fibrils are present. Very little periodic structure is noted in fibril imprints. × 22,000.

FIG. 13. Chromium-shadowed collodion replica of frozen section of adult human corium smeared directly onto glass slide. Showing imprints of a group of fibrils whose periods are in lateral register. × 36,000.

FIG. 14. Direct smear of the corium of a 2-day-old infant onto supporting film of specimen grid shadowed with chromium. Axial periodicity can be observed despite the presence of surrounding amorphous material. × 27,000.







(Gross and Schmitt: Human skin collagen studied with electron microscope)